

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application:

1-44. (Canceled)

45. (Currently Amended) A method of obtaining a full-length cDNA gene sequences comprising:

- (a) isolating a full-length mRNA;
- (b) attaching a double-stranded DNA tag sequence to the 5' end of the isolated mRNA wherein the DNA tag is covalently linked to a topoisomerase and wherein the topoisomerase is released upon attachment of the DNA tag, wherein the 5' strand of the DNA tag comprises a 5' tail, and wherein the 3' strand of the DNA tag comprises a 1 to 4 nucleotide overhang at its 5' end, wherein said overhang will complementary base pair with 1 to 4 nucleotides, respectively, on the 5' end of the isolated mRNA; and
- (c) synthesizing the cDNA using the DNA-tagged tagged mRNA as a template by contacting the DNA-tagged mRNA with a reverse transcriptase under conditions which permit reverse transcription,
so as to thereby obtain the full-length cDNA.

46-78. (Canceled)

79. (Currently Amended) A method of obtaining a full-length cDNA gene sequences comprising:

Applicants: Stewart Shuman, et al.

Serial No.: 10/666,486

Filed: September 19, 2003

Page 3 of 14 of Amendment in Response to May 5, 2008 Office Action

- (a) isolating a full-length mRNA by employing an affinity purification material;
- (b) decapping and dephosphorylating the isolated mRNA;
- (c) attaching a double-stranded DNA tag ~~sequence~~ to the 5' end of the decapped, dephosphorylated mRNA, wherein the DNA tag sequence is covalently linked to a vaccinia DNA topoisomerase and wherein the DNA tag comprises the sequences set forth ~~sequence shown in SEQ ID NO. 30 and 31 Figure 11 and is attached by vaccinia DNA topoisomerase;~~
- (d) synthesizing cDNA using the DNA-tagged ~~tagged~~ mRNA as a template by contacting the DNA-tagged mRNA with a reverse transcriptase under conditions which permit reverse transcription;
- (e) amplifying the synthesized cDNA, wherein the amplification primers comprise an anti-coding sequence of the tag sequence (5') and a gene specific sequence (3'); and
- (f) inserting the amplified cDNA into an expression vector.

80. (Previously Presented) The method of claim 45, wherein the mRNA is isolated by employing an affinity purification material.

81. (Previously Presented) The method of claim 80, wherein the mRNA to be isolated comprises an affinity purification tagged cap structure.

82. (Previously Presented) The method of claim 80, wherein the affinity purification tag is a biotin moiety, a chitin binding domain or a glutathione-S-transferase moiety.

Applicants: Stewart Shuman, et al.

Serial No.: 10/666,486

Filed: September 19, 2003

Page 4 of 14 of Amendment in Response to May 5, 2008 Office Action

83. (Previously Presented) The method of claim 80, wherein the affinity purification material comprises a solid support complexed with phenylboronic acid, streptavidin, avidin, chitin or glutathione.
84. (Previously Presented) The method of claim 83, wherein the solid support is magnetic beads or sepharose.
85. (Previously Presented) The method of claim 45, wherein the mRNA is isolated from plant cells or animal cells.
86. (Previously Presented) The method of claim 85, wherein the animal cells are mammalian cells or insect cells.
87. (Previously Presented) The method of claim 45, wherein the mRNA is decapped and dephosphorylated after isolation.
88. (Previously Presented) The method of claim 87, wherein the mRNA is decapped enzymatically or by chemical treatment.
89. (Previously Presented) The method of claim 88, wherein the enzyme is a pyrophosphatase.
90. (Currently Amended) The method of claim 88, wherein the chemical treatment is periodate oxidation and [[or]] beta elimination.
91. (Previously Presented) The method of claim 87, wherein the mRNA is dephosphorylated using alkaline phosphatase.

Applicants: Stewart Shuman, et al.

Serial No.: 10/666,486

Filed: September 19, 2003

Page 5 of 14 of Amendment in Response to May 5, 2008 Office Action

92. (Currently Amended) The method of claim 45, wherein the DNA tag ~~sequence~~ comprises a recognition site for a type I topoisomerase.
93. (Currently Amended) The method of claim 92, wherein the DNA tag ~~sequence~~ further comprises a recognition site for a site-specific restriction endonuclease.
94. (Previously Presented) The method of claim 92, wherein the type I topoisomerase is vaccinia DNA topoisomerase.
95. (Currently Amended) The method of claim 92, wherein the DNA tag ~~sequence~~ comprises the ~~double-stranded~~ sequences sequence set forth shown in SEQ ID NO. 30 and 31 Figure 11, wherein N in SEQ ID NO. 31 represents the 1 to 4 nucleotide overhang at the 5' end of the 3' strand, and of which each nucleotide is, independently, an adenosine moiety, a guanosine moiety, a cytosine moiety or a thymidine moiety.
96. (Canceled)
97. (Canceled)
98. (Currently Amended) The method of claim 45, further comprising amplifying the synthesized cDNA using amplification primers wherein the amplification primers comprise an anti-coding sequence of the tag sequence (5') and a gene specific sequence (3').
99. (Previously Presented) The method of claim 98, further comprising inserting the amplified cDNA into an expression vector.

Applicants: Stewart Shuman, et al.

Serial No.: 10/666,486

Filed: September 19, 2003

Page 6 of 14 of Amendment in Response to May 5, 2008 Office Action

100. (Currently Amended) The method of claim 45, wherein the DNA tag ~~sequence~~ is a linearized expression vector.